UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

March 10, 2003

MEMORANDUM

Subject: Efficacy Review for EPA Reg. No. 1677-129 / Oxysan
DP Barcode: D287155

From: Ian Blackwell, Biologist
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Applicant: Ecolab, Inc.

Formulation From Label:

Active Ingredient(s)	% by wt
Hydrogen peroxide	27.5
Inert Ingredient(s)	5.8
Total	100.0

I **BACKGROUND:** Ecolab, Inc., has submitted information, in accordance with FIFRA Section 6(a)(2), to report efficacy failure for the product, Oxy-Sept 333 (EPA Reg. No. 1677-129). The report states that this study "has not been performed meeting the Good Laboratory Practice Standards outlined in 40 CFR, Part 160."

The last accepted label (dated April 5, 2002 for Oxonia Active) indicates that the product is a sanitizer and "one-step" disinfectant for use on hard, non-porous surfaces. As a disinfectant, the product may be used in medical, institutional, industrial, animal care, and commercial environments. EPA Reg. No. 1677-129 is associated with the following product names:

Source	Product Name
Bean Sheet	Oxysan
Request for 6(a)(2) Screen	Oxy-Sept 333
Correspondence from Registrant	Oxy-Sept 333
Product Label	Oxonia Active

The study was conducted by Ecolab, Inc. The MRID Number is 457506-01.

II **Use Directions**

The product is designed to be used for disinfecting hard, non-porous surfaces such as floors, walls, tables, chairs, counter tops, bathroom fixtures, sinks, shelves, racks, carts, refrigerators, and coolers. The product may be used to disinfect tile, linoleum, vinyl, asphalt, porcelain, plastic, stainless steel, or glass. Directions on the last accepted label (dated April 5, 2002) provided the following information regarding preparation and use of the product as a disinfectant: Prepare a use solution by diluting 4 ounces of product in 8 gallons of water. Pre-clean heavily soiled areas. Apply the use solution using a mop, cloth, sponge, brush, scrubber, or coarse spray device or by soaking. Wet all surfaces thoroughly. Allow the surfaces to remain wet for 10 minutes. Remove the use solution and entrapped soil with a clean wet mop, cloth, or wet vacuum pickup.

III **Agency Standards for Proposed Claims**

1 General or broad-spectrum efficacy claims. Label claims of effectiveness as a "general disinfectant" or representations that the product is effective against a broad spectrum of microorganisms are acceptable if the product is effective against both Gram-positive and Gram-negative bacteria.

A Test requirements. Use the AOAC Use-Dilution Method or the AOAC Germicidal Spray Product Test as in (a)(l). Sixty carriers must be tested against each of both *S. choleraesuis* and *S. aureus* with each of 3 samples, representing 3 different batches, one of which is at least 60 days old. (120 carriers per sample; a total of 360 carriers.)

B Performance requirements. To support products represented in labeling as "disinfectants", killing on 59 out of each set of 60 carriers is required to provide effectiveness at the 95% confidence level.

2 Hospital or medical environment efficacy claims. Label claims for use of disinfectants in Hospital or medical environments are acceptable only for those products that are effective for general or broad-spectrum disinfection and additionally against the nosocomial bacterial pathogen *Pseudomonas aeruginosa*.

A Test requirements. Employ the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products Test as in (a)(l). Sixty carriers must be tested against each of *S. choleraesuis*, *S. aureus*, and *Pseudomonas aeruginosa* ATCC 15442 with each of 3 samples, representing, one of which is at least 60 days old. (180 carriers per sample; a total of 540 carriers.)

B Performance requirements. Same as in (1)(B) above.

IV Comments on the Submitted Efficacy Studies

1 MRID 457506-01: "Oxy-Sept 333 Use Dilution Methods," by Joshua Magnuson. Study conducted at Ecolab, Inc., Research and Development Center, Mendota Heights, Minnesota. Study completion date – August 22, 2002.

This study was conducted against *Pseudomonas aeruginosa* (ATCC 15442) and *Staphylococcus aureus* (ATCC 6538) in the presence of a 5% organic soil load (fetal bovine serum). Two lots (Lot Nos. 10623-103A and 10623-103B) of the product, Oxy-Sept 333, were tested using the AOAC Use-Dilution Method as described in the AOAC Methods of Analysis, 15th Edition, 1990. Both product lots were at least 60 days old at the time of testing. A use solution was prepared by adding 7.8 mL of product into a final volume of 2.0 L of 500 ppm synthetic hard water (equivalent to 4 ounces/8 gallons). The diluent used was prepared and titrated (at 490 ppm) on the day of testing. During the preparation of the test organisms, the addition of 5% organic soil and 0.005% sodium stearate was performed prior to vortexing the test organism. Sixty (60) stainless steel carriers were immersed in a 48±4 hour broth culture and soaked for 15 minutes. The

carriers were removed and placed on filter paper within a Petri dish to dry at 37±2°C for 40±1 minutes. After the incubation period, the carriers were removed from the incubator and used for testing within 60 minutes. The carriers were placed into test tubes of the use solution and exposed for 10 minutes at 20±2°C. The carriers were transferred to Letheen Broth, then incubated at 37±2°C for 48±4 hours. Controls included numbers control, sterility, neutralizer effectiveness, and confirmation of the challenge microorganism.

Note: The initial data were discounted because the neutralizer was reported to be insufficiently effective in neutralizing the product. The MRID [page 8] states that the Letheen Broth "did not adequately neutralize the product and therefore was detrimental to the test system."

2 [Appendix II of MRID 457506-01] "Comparison of the Use-Dilution Disinfectant Efficacy and Non-food Contact Sanitizing Efficacy of Oxy-Sept 333 with Current LAS and with Replacement LAS," by Joshua Magnuson and Linda Grieme. Study conducted at Ecolab, Inc., Mendota Heights, Minnesota. Study completion date – August 22, 2002.

The re-test, described in Appendix II of MRID No. 457506-01, was initiated on August 14, 2002. The re-test was conducted against *Pseudomonas aeruginosa* (ATCC 15442) and *Staphylococcus aureus* (ATCC 6538) in the presence of a 5% organic soil load (fetal bovine serum). Two lots of the product, Oxy-Sept 333, were tested using the AOAC Use-Dilution Method as described in the AOAC Methods of Analysis, 15th Edition, 1990. A use solution was prepared by diluting 7.8 mL of the product into a final volume of 2 L of 500 ppm synthetic hard water (titration results not provided). The criteria for the re-test (including the organic soil load) appear to be the same as the initial test except the neutralizer is replaced by 9.0 mL of Sodium Thioglycollate. (The full details of the re-test are not provided.) The re-test used two lots of product, Lot No. 10623-103A "with BIO-SOFT S-100 (current LAS)" and Lot No. 10623-103B "with BIO-SOFT S-101 (new process LAS)."

Note: Appendix II also included a study entitled "Non-Food Contact Sanitizer Method: Sanitizer Test for Inanimate, Non-Food Contact Surfaces", which is not relevant to the disinfectant claims being discussed in this efficacy report.

V Results

MRID Number	Organism	No. Exhibiting Growth/Total No. Tested		Viability (CFU/mL)
		Lot No. 10623-103A	Lot No. 10623-103B	
457506-01	*Staphylococcus aureus* Test Date: 07/23/02 Test Date: 08/14/02	 0/60 0/60	 0/60 0/60	 1.5×10^6 1.3×10^6
457506-01	*Pseudomonas aeruginosa* Test Date: 07/23/02 Test Date: 08/14/02	**3/60** 1/60	**3/60** 0/60	8.1×10^5 4.6×10^6

VI Conclusions

1 The submitted efficacy data do not support the use of the product, Oxysan, as a general disinfectant when tested against *Pseudomonas aeruginosa* (ATCC 15442) and *Staphylococcus aureus* (ATCC 6538) in the presence of a 5% organic soil load (fetal bovine serum) on hard, non-porous surfaces for a contact time of 10 minutes at a dilution of 4 ounces of product to 8 gallons of water. The problem with this study is that the product failed 3/60 times when tested against *Pseudomonas aeruginosa* (ATCC 15442). **However,** the report states that this study "has not been performed meeting the Good Laboratory Practice Standards outlined in 40 CFR, Part 160." Although the lab states that the study was not conducted in accordance with GLP guidelines, the report does not state what the GLP deficiencies were.

2 Review of the 6(a)(2) data submission indicate the ambiguity of the efficacy data (MRID No. 457506-01) submitted by the applicant regarding the use of the product, Oxy-Sept 333, as a disinfectant against *Pseudomonas aeruginosa* when tested in the presence of a 5+% organic soil load (fetal bovine serum and dried soap scum) on hard, non-porous surfaces for a contact time of 10 minutes. In the initial test, growth was observed in the subcultures of 3 of the 60 carriers for both lots of the product tested (i.e., Lot Nos. 10623-103A and 10623-103B). The report states that the Letheen Broth "did not adequately neutralize the product and therefore was detrimental to the test system." The report did not include the data from neutralization effectiveness testing. Failure of the neutralizer performance, however, would not explain the observation of test organism

survival. In fact, if the neutralizer failed, the product would have a longer contact time and would have potentially killed more test organisms, making the product's efficacy artificially too high. When the study was repeated with Sodium Thioglycollate as the neutralizer, growth was observed in the subcultures of only 1 of the 60 carriers for one of the two product lots tested (i.e., Lot No. 10623-103A). Again, the report did not include the data from neutralization effectiveness testing.

VII Recommendations

1 The submitted data cannot currently be used to support a labeling change for EPA Registration Number 1677-129. If this study were accepted, it would mean that the registrant would have to at least temporarily remove labeling claims of this product being an effective disinfectant against *Pseudomonas aeruginosa* (ATCC 15442). Although the product failed the disinfectant testing against *Pseudomonas aeruginosa* (ATCC 15442), there are issues that the testing facility and/or registrant need to clarify:

A In what specific ways was this study not conducted in accordance with GLP guidelines?

B Please provide the results of the neutralizer screening test discussed on page 8 of 27 of the report (MRID Number 457506-01).

C How is it that the lab feels that a neutralizer failure would result in an increase in the bacterial count of the slides treated with 1677-129? It seems to EET/PSB that a failure of the neutralizer would **reduce** the bacterial count of treated slides.

2 Review of the 6(a)2 data submission support continued label claims for efficacy of the product, Oxy-Sept 333, as a disinfectant against *Staphylococcus aureus* for a contact time of 10 minutes.